Life History Observations on *Thrips florum* (Thysanoptera: Thripidae) Infesting Gardenia in Hawaii, and a Comparison of the Humidity Requirements for *T. florum* and *Frankliniella occidentalis*

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Abstract. *Thrips florum* Schumtz is a common pest of gardenia (*Gardenia jasminoides* Ellis) flowers in Hawaii. Typically hundreds of thrips infest each flower, and their feeding and egg laying punctures result in a brown discoloration of the white petals. The life cycle and biology of this pest was studied on gardenia flowers to facilitate proper timing of control procedures. This is possibly the first report on the developmental biology of this species. In laboratory tests, the development period from egg to adult female required a minimum of 17 days at 20°C and 11 days at 24°C. Significant proportions of second instars, propupae and pupae were found off the flower and resting on the bottoms or sides of rearing containers. Difficulty in rearing insects led to the observation that adults could not tolerate even short exposures to low or moderate levels of relative humidity (RH). In controlled humidity tests, only 19% of *T. florum* survived a 24-hour exposure to 92.5% RH, whereas this level was tolerated by >90% of western flower thrips, *Frankliniella occidentalis* (Pergande). In field studies, the number of *T. florum* infesting gardenia blossoms in a 2-acre gardenia planting typically exceeded 200 adults per flower. Removing all open gardenia blossoms from the field once per week was associated with large reductions in the thrips population and an elimination of noticeable damage to flower buds.

Introduction

The *Thrips hawaiiensis* (Morgan) species-group is currently represented in Hawaii by two species: *T. hawaiiensis* and *Thrips florum* Schumtz (Palmer and Wetton 1987; Nakahara 1985). These two species are morphologically very similar, and *T. florum* was considered a synonym of *T. hawaiiensis* until re-validated by Nakahara (1985). For this reason, published information relating to the distribution, host range and biology of these two species may be confounded. Individuals within the *T. hawaiiensis* species-group are commonly found on a wide variety of tropical flowers, fruits and seed crops within the Oriental and Oceanic Regions to the Caribbean Basin, from Japan and Hawaii to New Zealand and the Tuamotu Islands (Palmer and Wetton 1987). A world map showing confirmed distributions for *T. hawaiiensis* and *T. florum* is given in Palmer and Wetton (1987). *T. florum* is also reported from Costa Rica, Guatemala and Florida (Mound and Marullo 1996; Nakahara 1994). In Hawaii, *T. florum* is confirmed from Oahu, Maui and Hawaii Island (personal communication, Tom Watanabe, APHIS, Honolulu, 2003). Host associations for *T. florum* based on museum records at the US National Museum (Washington, DC) include *Bassia* sp., *Couropita* sp., *Fagraea berteriana* Benth.,*Gardenia jasminoides* Ellis, *Hibiscus* sp., *Hedychium* sp., *Lantana camara* L., *Jasminum* sp., *Strongylodon* sp., *Camillia sinesis* (L.) Kuntze (Steve Nakahara, personal communication, 2003).

Although thrips in the *T. hawaiiensis* complex are considered to serve a beneficial role as pollinators of oil palm and *Cosmos* (Varatharajan *et al.* 1982; Syed 1979), they are serious

pests of coffee, mangoes, citrus, apples, pears, passion fruit, roses and bananas (Palmer and Wetton 1987; Abraham *et al.* 1970; Ananthakrishnan 1971; Swaine and Corcoran 1975; Lee and Wen 1982; Srivastava and Bhullar 1980). An extensive list of hosts is found in Zimmerman (1948). Larvae and adults feed within flowers on pollen and cell sap, causing discoloration, malformed buds, and loss of fruit set. They also scar the surface of young fruit. Although both species have been collected from gardenia flowers in Hawaii (personal communication, Dick M. Tsuda, 2003), there is no published information indicating which species is more common on this host. A single gardenia flower may contain hundreds of dark-colored adults (females of *T. florum* and/or *T. hawaiiensis*). The light colored males are also present, but in lower numbers. Adults outnumber larvae in recently opened flowers, but after several days, larvae typically outnumber adults, due to a combination of adult migration and the hatching of eggs deposited within petals and other flower parts. When thrips populations are high, flowers become infested in the bud stage, and the outer (white) petals develop a brown discoloration due to thrips feeding and oviposition. Because gardenia flowers have a relatively short vase-life (about 1 week), flower buds, not open flowers, are typically picked for commercial sale.

Currently in Hawaii, gardenia is produced commercially on an estimated 3.6 acres (Anonymous 2002). Acreage may soon increase because of a new federal regulation (published in February 2003) that permits gardenia flowers to be exported to the U.S. mainland for the first time since 1948 (Anonymous 1948; Anonymous 2002). Although the previous ban was due to the risk of green scale (*Coccus viridis* (Green)) infestation, flower inspections mandated under the new quarantine protocol will include searches other pests such as thrips. *T. florum* (but not *T. hawaiiensis*) is considered an "actionable" pest by APHIS (Animal and Plant Health Inspection Service) (personal communication, Tom Watanabe, APHIS, Honolulu, 2003). Therefore, pest management practices for thrips control will be of key importance for meeting quarantine standards and avoiding quarantine rejections.

There is no previous report dealing with the developmental biology of *T. florum*. An understanding of the life cycle on gardenia flowers may lead to improved strategies for control, particularly with regard to timing of pesticide applications and field sanitation measures (removal of unharvested flowers upon which thrips reproduce). Our goals in this study were to: (1) determine the length of the life cycle of *T. florum* on gardenia blossoms at 20 and 24°C; (2) compare the survival of *T. florum* and *Frankliniella occidentalis* (Pergande) at various levels of relative humidity; and (3) gather preliminary data on the potential of field sanitation (removal of open flowers) as a cultural control method for *T. florum* infesting gardenia flowers.

Materials and Methods

Laboratory studies were carried out at the Waiakea Experiment Station (Hilo, Hawaii) between June 2000 and April 2001. *T. florum* used in all studies were field collected from a 2-acre planting of gardenia at Hualalai Flower Farms, Holualoa, Hawaii, which was also the site used for the sanitation trial (described below). Voucher specimens of *T. florum* associated with life cycle studies were identified by Steve Nakahara (USDA-ARS Systematic Entomology Laboratory) and Dick M. Tsuda (University of Hawaii, Manoa), and will be deposited in the Entomology Museum, Gilmore Hall, University of Hawaii at Manoa. The majority of the voucher specimens (>95%) were determined to be *T. florum*, but two specimens were determined as *Thrips* sp. nr. l*eeuweni* Priesner, an exotic species recently detected in Hilo, Hawaii (Steve Nakahara, personal communication, 2003). *F. occidentalis* used in relative humidity tests were females <2 wk old reared in the laboratory at ambient temperature on a diet of green beans and bee pollen. Flowers used in trials were from Holualoa

or from plantings in the Hilo area.

Life cycle on gardenia flowers. Gardenia flowers were picked as mature flower buds at the "white line" stage (just prior to the opening of flower buds, after expansion of the bud caused the greenish outer petals to slide over and expose a portion of the underlying white petals). The outer petals and tips of flowers were removed to eliminate *T. florum* eggs deposited in the outer petals. In preliminary tests, contamination from this source was discovered to be significant. Flowers were provided with water via florist's tubes and placed individually into 1.5-liter snap-top white plastic containers (14-cm diameter by 15-cm tall). Containers were unventilated for the test at 20°C; for the test at 24°C, each container was ventilated via silkscreen inserts (7.5 cm diameter) glued into the lid. The ventilated area was loosely covered with a Petri dish to keep humidity high. This alteration of lids improved rearing success. Adult female *T. florum* were transferred into each container using a motorized aspirator attached to a 65-ml black aspirator vial. To infest flowers, we used 80 and 40 females per container for the tests at 20°C and 24°C, respectively. Females were then removed on Day 1 (24 hours after being added) using an aspirator and a brush.

Containers were stored in a Conviron environmental growth chamber (Model CMP 3244, Controlled Environments Inc., Pembina, North Dakota). Lighting in the chamber was supplied between 6:00 A.M. and 6:00 P.M via four 20-watt "Cool White" fluorescent bulbs (two lights above each of the two shelves holding containers). Temperature and relative humidity were monitored using a data logger (HOBO H8 Pro, Onset Computer Corp., Bourne, MA) placed inside a container with a flower but no thrips. These loggers reportedly have an accuracy level of ±0.3°C and 3% RH. The average temperature and RH in the first experiment (ranges given in parentheses) were 19.8°C (19.0 to 21.3) and 103.5% RH (99.4 to 103.9) [*sic*]. In the second experiment, the average temperature was 23.75°C (22.98 to 24.42) and the RH was 93.4% (85.7 to 101.5). To simplify the discussion, temperatures used in the first and second experiments are referred to as 20 and 24°C, respectively. Every 7-8 days, a new gardenia flower (with florist's tube) was added to each container. The old flower was left in place.

Development of thrips life stages in containers was assessed after washing thrips out of blossoms and containers (separately) using a rinse of 60% alcohol. Thrips removed in this fashion were collected by sieving onto silkscreen mesh and counted under a dissecting microscope. The two larval instars were separated on the basis of size and body color (bodies of 1st instars were without color while second instars were generally pinkish). Propupae were distinguished from pupae by the wing buds of the former. One container was processed each day throughout the duration of tests, except in the test at 20°C, extractions began on the third day, and the data presented for Days 21 and 22 represent averages from 3 and 7 containers, respectively. To determine natural levels of infestation in the flowers used for tests, we also washed thrips from control flowers prepared in the same way but not artificially infested. Nine control flowers (1 flower per container) were set up at the beginning of each test and 7-8 days later, at the time that an additional flower was added to each container. Seven days after set-up, insects were washed from control flowers and counted.

Survival of *T. florum* and *F. occidentalis* at various relative humidity regimes. We measured the mortality of adult female *T. florum* and *F. occidentalis* exposed to various constant relative humidity (RH) environments for 24 hours at 25°C within plastic Petri dishes (no food provided). *F. occidentalis* was chosen as a reference species because it has a wide host range and is found in many different kinds of environments. The RH environments were created using saturated salt solutions placed in the bottom of 1.5-liter plastic containers (14-cm diameter by 15-cm tall). The salt solutions (and associated % RH at equilibrium based on published literature) were potassium carbamate (97.5%), potassium nitrate (92.5%), sucrose (85.0%), sodium chloride (75.5%), and potassium carbonate (43.0%)

(Winston and Bates 1960). One hundred percent RH was obtained using distilled water. Equilibration of salt solutions with the atmosphere inside containers required <1 hr and was verified using HOBO data loggers that were placed inside containers on top of a 150-ml beaker. These data loggers were also used to verify that experiments were carried out at ~25ºC. Based on data from these data loggers, the average temperature during the 24-hour exposure periods was 24.8ºC (24.61–24.96).

Containers were kept covered with a solid top until needed for tests, at which time specially designed covers containing test insects were quickly slipped over in place of the solid covers. The special cover was made by gluing a plastic Petri dish (9-cm diameter) from which the bottom had been removed over a 15-cm plastic Petri dish lid that contained a 4.5-cm diameter silkscreen-covered hole in the center. The silkscreen insert prevented thrips from escaping the 9-cm Petri dish but allowed gas exchange between the Petri dish above and the 1.5 liter container holding the saturated salt solution below. A moistened brush was used to transfer 10 *F. occidentalis* or 20 *T. florum* adult females into each Petri dish. Petri dishes holding thrips were sealed with Parafilm® (American National Can, Chicago, IL.). The two species were tested separately, approximately one month apart. However, on each day that tests were conducted, all humidity levels were tested in side-by-side comparisons using an equal number of replicates of each RH level. Containers with thrips set up in this fashion were kept within an environmental chamber (described previously) at 25ºC for 24 hours, and then living and dead thrips were counted with the aid of a dissecting microscope.

Thrips abundance in relation to field sanitation. At a 2-acre gardenia planting in Holualoa, Hawaii, we tested sanitation as a potential control for *T. florum*. Once per week between 29 December 1999 and 9 February 2000, we picked and removed from the field all open gardenia blossoms. Sanitation measures (~once per wk, carried out by the grower) resumed 29 March through May 4, 2000. To measure the effect on the thrips population, we extracted adult thrips from 10 newly-opened gardenia flowers, 5 flowers in the upper part of the field and 5 from the lower section, picked each week just prior to performing field sanitation. These randomly selected flowers were placed individually into Ziploc bags and taken back to the laboratory in a cooler. Thrips were separated from flowers by washing flowers in 60% alcohol and sieving over silkscreen. Adult males and females were separated on the basis of color (males with lighter bodies) and counted with the aid of a dissecting microscope.

Results

Life cycle on gardenia flowers. Due to the structure of gardenia flowers and the many hiding places they afforded to thrips, we were not successful in removing all of the adult female thrips used for infesting flowers after the end of the 24-hour infestation period. We removed 96 and 97.5% of the females used for infesting containers held at 20 and 24°C, respectively. This is based on the average number of females recovered from extractions prior to the time that the progeny thrips within the containers first developed into adults (=Day 15 at 20° and Day 9 at 24°). Newly emerged adult females were teneral and light colored in contrast to the brown, older females. This allowed us to distinguish them from any residual *T. florum* females used for infesting flowers.

At 20ºC, first instar larvae, second instar larvae, pupae, adult males and adult females were first observed 6, 7, 12, and 15 and 17 days after artificial infestation, respectively (Fig. 1). The number of insects recovered per day beginning with the 6th day after flowers were infested varied from 27 to 141, which corresponds to an average of 0.3 to 1.8 progeny/female for the 80 female thrips used to infest each flower. At 24ºC, first instar larvae, second instar larvae, pupae, adult males and adult females were first observed 2, 4, 8, 9 and 11

Figure 1. Life stages of *T. florum* recovered from gardenia flowers held at 20ºC. AF, adult female; AM, adult male; P, pupa; PP, propupa; LII, second-instar larva; LI, first-instar larva.
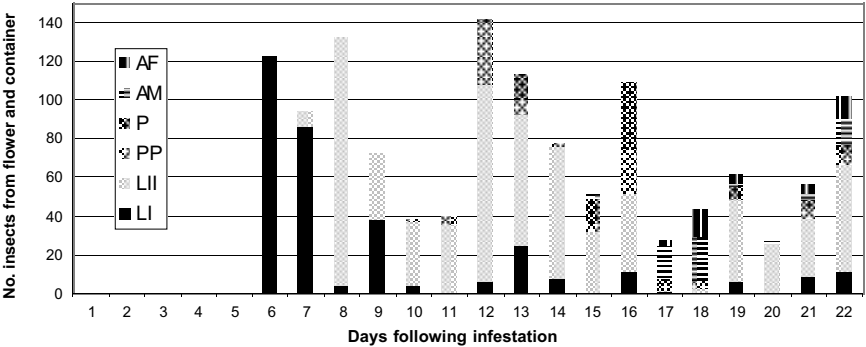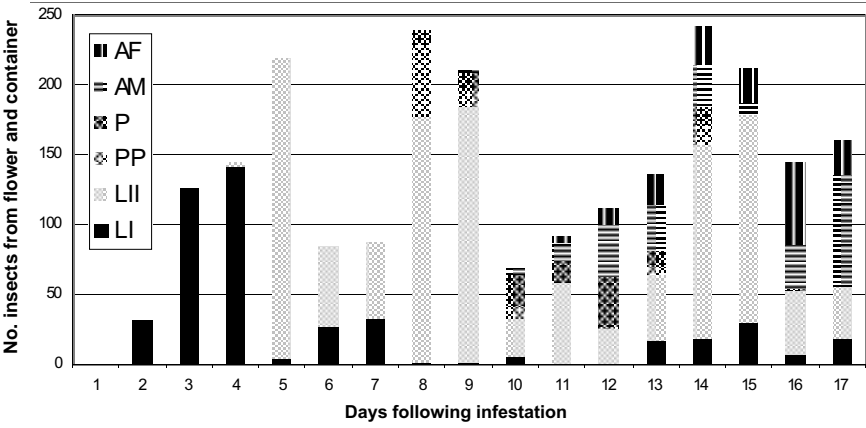

Figure 2. Life stages of *T. florum* recovered from gardenia held at 24ºC. For key to abbreviations, see Fig. 1 legend.

days after infesting, respectively (Fig. 2). The number of insects recovered per day beginning with the 3rd day after flowers were infested varied between 69 and 238, which corresponds to an average of 1.7 to 6.0 progeny/female for the 40 female thrips used to infest each flower. Significant numbers of insects of all life stages except first instar larvae were found off the flower on the sides and bottoms of the rearing containers in all tests (Fig. 3 shows data for 24ºC). At 24ºC, the percentage of insects washed from the rearing container after the flower was removed was 2.8, 49.5, 34.0, 47.1, 20.9 and 23.8% for first instars, second instars, propupae, pupae, adult males and adult females, respectively. At both temperatures, a significant percentage of second instars exhibited delayed development. This is evidenced by large numbers of second instars present in flowers during the majority of the sampling periods.

Figure 3. Life stages of *T. florum* washed from rearing containers held at 24ºC after infested flowers were removed.
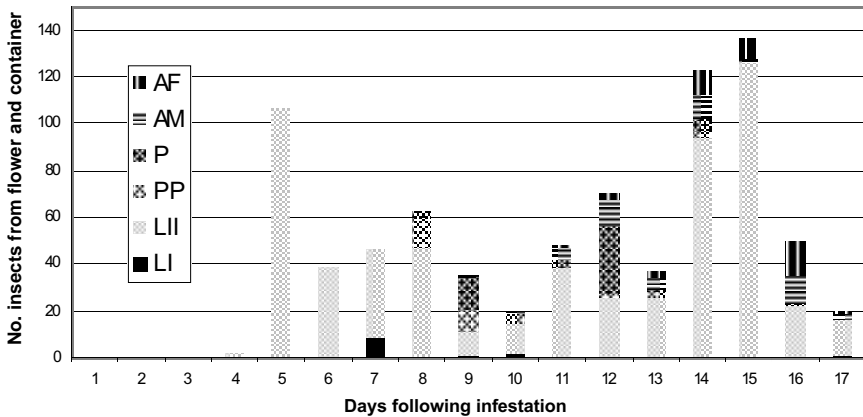

Survival of *T. florum* and *F. occidentalis* at various regimes of relative humidity. Relative to *F. occidentalis*, the RH requirements of *T. florum* were remarkably high (Fig. 4). At 100% RH, 24-hour survival of *T. florum* and *T. occidentalis* was 94.8 and 98.1%, respectively. However, at RH values of 97.5 and 92.5%, survival of *T. florum* was only 66.3 and 18.9%, respectively. In sharp contrast, at these same RH values, survival of *F. occidentalis* was 98.1 and 90.5%, respectively.

Thrips abundance in relation to field sanitation. On 29 December, at the time that sanitation procedures were begun, populations of thrips were high, averaging over 200 adult thrips per flower (Fig. 5). Populations reached a low of 57 adult thrips per flower on 16 February, one week after discontinuing sanitation measures. Over the next six weeks, populations gradually increased, until reaching a peak of >400 adults per flower on 29 March, at which time sanitation measures were begun again. Populations again declined in association with sanitation from 29 March until our last sampling on 4 May, at which time we measured an average of 78 adult thrips per flower.

Discussion

In our life cycle studies, development time of *T. florum* from egg to adult female required 17 days at 20°C and 11 days at 24°C. Murai (2001) found that the average developmental time of *T. hawaiiensis* from egg to adult (either sex) was 13.0 days at 20°C and 8.9 days at 25°C. Murai carried out this work in Japan using insects reared individually on tea pollen. A Chinese scientist, Cheng (1985), studied the life cycle of *T. hawaiiensis* on citrus blossoms, and found that development time from egg to adult required 10.9 days at 25°C. These results agree closely with our own. In our study at 20°C, first instars were not found until 6 days after infestation. The high RH (100%) within the unventilated rearing containers may have inhibited egg hatch for several days. In an earlier run of the same experiment, containers tested with ventilated lids (7.5-cm diameter silkscreen inserts) resulted in an average RH of 87.5% in the container and recovery of first instar larvae 4 days after infestation. We switched to the unventilated containers because they resulted in both higher survival of the female thrips used to infest flowers and a greater number of progeny. However, droplets of

Figure 4. Percent survival (± SEM) of adult female thrips as a function of relative humidity.
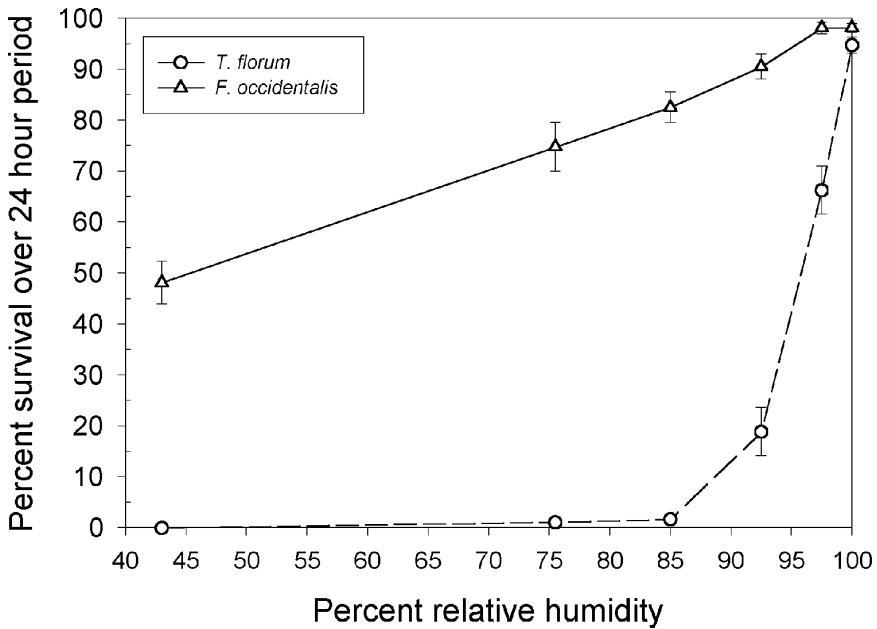
condensation that formed inside these containers apparently interfered with successful molting, causing bloating and death. For the test at 24°C, we achieved very high humidity without excessive condensation using container tops ventilated with a 7.5-cm diameter mesh insert loosely covered with a Petri dish lid.

Our observation that adult female thrips required very high humidity for survival motivated our second study using saturated salt solutions. The low survival of *T. florum* at relative humidity levels <97.5% is quite surprising, but correlates with field observations for thrips in this species complex. Sakimura and Krauss (1944) published a survey of thrips species present on Maui and Molokai in which they noted that *T. hawaiiensis* preferred wet or shady habitats, in contrast to other flower thrips such as *Frankliniella* sp. Presumably flower structure is one reason gardenia is an excellent host for *T. florum*, which typically feeds deep within the flower between petals that are tightly appressed. Undoubtedly relatively humidity is high in the microclimate between flower petals.

The results of the field trial suggest that sanitation (removing unharvested, open flowers) is a useful technique for reducing populations of adult thrips. Observations made in the field indicate that second instar larvae are capable of migrating between flowers, as they are sometimes found on flower buds along with a small number of adult females (particularly when thrips populations are high). In the life cycle study at 24°C, a significant proportion of second instar larvae were found away from the flower within the rearing container 5 days after artificial infestation. The behavior of leaving the flower was probably associated with pupation, as most thrips species drop to the ground for pupation during the latter part of the second instar. Based on this observation and assuming that the rate of thrips development at 24°C equals or exceeds the maximum rate of development under field conditions, open

Figure 5. Average number (± 1 SEM) of adult thrips per gardenia flower at a 2-acre gardenia farm in Holualoa, Hawaii (west Hawaii Island) in relation to sanitation measures.
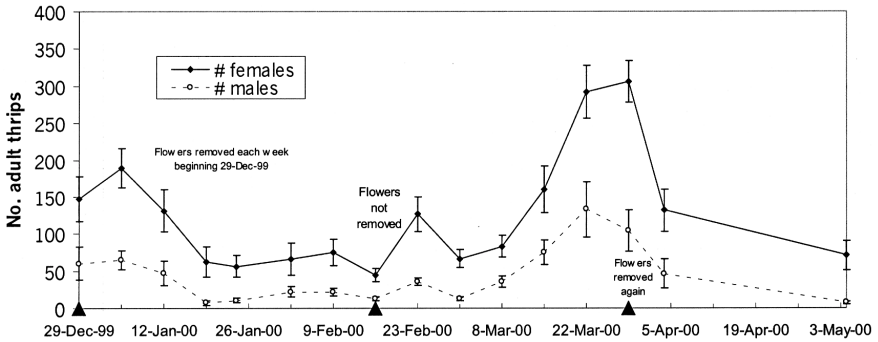
flowers should be removed from the field at least once every 5 days to ensure that insects developing within flowers will not escape collection.

In consideration of the dispersal ability of adult thrips, we did not set up a concurrent control when testing the effect of the sanitation treatment. The drop in population levels associated with flower removal was not likely a coincidence and it apparently was not correlated with the availability of flowers in the field. Flower production records kept by the grower indicate that production is relatively low from January-March, but increases 5-fold during the months of April and May. Based on this information, the first population decline occurred during the period when flower production was low, while the second decline occurred during the period when flower production was very high. During field sanitation procedures, outer petals on flower buds had a brown discoloration when thrips populations were high due to feeding and egg laying by adult females. However, this damage did not occur when populations were low. Recently opened gardenia flowers have a very strong floral scent and are highly attractive to adult thrips. Based on this observation, I hypothesize that good sanitation procedures would greatly reduce the incidence of thrips on unopened flowers (the marketable commodity).

Both *T. hawaiiensis* and *T. florum* have been collected from gardenia flowers in Hawaii. With few exceptions, females of *T. florum* have dark brown bodies and 7-segmented antennae (Palmer and Wetton 1987). Rarely an individual may have 8 segments on one of the antennae. Females of *T. hawaiiensis* are either entirely brown, or have a brown abdomen with a lighter colored head and thorax. *T. hawaiiensis* have antennae with either 7 or 8 segments or one antenna of each type. However, female *T. hawaiiensis* having 7 segments usually have bi-colored bodies, a combination of features that helps to separate it from *T. florum*. The easiest way to separate the two species is to examine the veinal setae on the fore wing scale (lobe like base of the wing with a row of five setae). In *T. florum* the apical seta is shorter than the penultimate seta and in *T. hawaiiensis* the apical seta is longer than the penultimate seta (Steve Nakahara, personal communication, 2003). Males are more easily and accurately identified, but are collected less frequently (Mound and Kibby 1998). *Thrips* sp. near *leeuweni* Priesner is a recent introduction that is found primarily in the flowers of *Fagraea berteriana* Benth. on Hawaii Island. This species has also been found in gardenia on several occasions, including gardenia flowers from Holualoa used in our study. The coloration of antenna, body and wings are similar to those of *T. florum*, and it is possible

that this species may displace *T. florum* in gardenia over time (Steve Nakahara, personal communication, 2003).

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